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Inhibitory Effect of *Buthus martensi Karsch* Extracts on α-Glucosidase Enzyme

Eun-Ok Kim and Shin-Duk Kim*
Department of Biological Engineering, Seokyeong University, Seoul 136 - 704, Korea

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While searching for α-glucosidase inhibitors, the active compound was found in a methanol extract of *Burthus martensi Kirsch*. The separation of the active compound was performed using various chromatography methods and the physico-chemical properties of the purified compound were characterized. The compound showed very potent inhibitory activity against α-glucosidase with an IC_{50} value of 5.3 μg/ml. Lineweaver-Burk plot indicated that its inhibition of α-glucosidase was competitive.

key words: α-glucosidase inhibitory activity, *Buthus martensi Karsch,* Competitive inhibitorIntroduction

Introduction

The α-glucosidase is essential to carbohydrate digestion because only monosaccharides are readily taken up from the intestine and all other carbohydrates have to be broken down enzymatically in the intestine before they can be absorbed. The other cellular glucosidases are known to be vital for the processing of Asn-linked glycoproteins and glycolipids (Kornfeld and Kornfeld, 1985; Asano, 2003), which are involved in various biological reactions such as immune responses, metastasis of cancer and viral infections (Fischer *et al*., 1995).

The inhibitors of the α-glucosidase inhibitors exhibit a high promise as therapeutic agents for the treatment of metabolic disorder such as diabetes mellitus, obesity and hyperglycemia (Martin *et al*., 1991). They also exerted the antiretroviral potency by inhibiting human immunodeficiency virus replication (Sunkara *et al*., 1987; Mehta *et al*., 1998; Chapel *et al*, 2006; Elbein, 1987) and antimetastatic activity by inhibiting platelet aggregation of metastatic cells as well as reducing adhesion of tumor cells to the vascular endothelium (Spearman *et al*., 1991; Watson *et. al*., 2001). The α-glucosidase enzyme, therefore, has been identified as a target with multiple therapeutic applications (Jacob, 1995; Tifft and Proia, 2000).

Historically, the screening of the extracts from the oriental medicines has yielded novel natural products that are potentially bioactive. While searching for the a-glucosidase inhibitors, the active compound was discovered from the methanol extract of the Asian scorpion, *Burthus martensi Kirsch* that has been used as a drug for the treatment of immune related disease in traditional oriental medicine. In this paper, the isolation and the partial characterization of α-glucosidase inhibitory compound from *B. martensi Karsch* have been described.

Materials and Methods

Materials

p-nitrophenyl (PNP)-α-D-glucopyranoside and brewers yeast α-glucosidase were purchased from Sigma, USA. *B. martensi Karsch* was purchased from a local herbal drug store.

Enzyme assays

The enzymatic activity of α-glucosidase was assayed chromogenically by the production of p-nitrophenol using p-nitrophenol-α-D glucopyranoside as substrate.

To a 96-well plate with a maximum volume per well being 300 μl, the assay solutions and the potential inhibitors were added as follows; 20 μl of 0.1 M phosphate buffer (pH 7.0), 20 μl inhibitor, 10 μl enzyme, 10 μl of substrate and 40 μl of methanol. The solutions were mixed and then incubated at 37°C for 30 min. Following incubation, the reaction was terminated by the addition of

*To whom the correspondence addressed
16-1 Jungneung-Dong, Sungbuk-Ku, Seoul 136 - 704, Korea.
Tel: 02-940-7171; Fax: 02-919-0345; sdkim@skuniv.ac.kr

3 volumes of NH_4OH solution. The p-nitrophenol liberated was determined with a microplate reader model 550 (Bio-Rad, CA, USA) at 405 nm.

Various modifications of the standard assay mixture were made in order to test the effects of the inhibitory compound. In some cases, enzyme, buffer, and varying amounts of the inhibitory compound were mixed and aliquots were removed at various time intervals over a 1 h period, and the reaction was initiated by the addition of the substrate. In other experiments, the substrate concentration was gradually increased at fixed concentrations of the compound in order to determine the type of inhibition.

The percentage inhibition was calculated by the formula $(A\text{-}B)/A \times 100$, where A is the p-nitrophenol resulting from the enzymatic hydrolysis without inhibitor and B is that in the presence of the inhibitor. The IC_{50} value is the concentration of inhibitor at 50% of enzyme activity.

The enzyme inhibition mode was determined from the Lineweaver-Burk plot.

Isolation of inhibitory compound

About 2000 dried adults of *B. martensi Karsch* were purchased from local market. The scorpion bodies were frozen and ground into fine powder. The ground material was suspended with methanol and centrifuged at room temperature. The supernatant was concentrated at reduced pressure and was extracted with butanol. The butanol layer was concentrated to dryness *in vacuo*. The resulting oily residue was dissolved in a small amount of a solvent mixture (butanol-methanol-water = 4 : 1 : 2) and applied on a column of silica gel (2.5 × 50 cm, kiesel gel 60, Merck co) which was packed and equilibrated with the same solvent mixture used to dissolve the crude extract. The column was eluted with the same isocratic solvent system. Fractions containing the active compound were taken to dryness, dissolved in methanol and subjected to a Sephadex LH-20 column developed with MeOH. TLC analyses were conducted on Silica Gel plates (E. Merck 60 F_{254}, n-propanol : 1 N NH_4OH = 7 : 3). The compounds were revealed using a 5% solution of H_2SO_4 in EtOH followed by heating. Final purification was achieved by HPLC (C_{18} reverse phase column, acetonitrile : water = 28 : 72 as an isocratic solvent system, UV detection at 206 nm) to give a single compound.

Results and Discussion

Isolation and purification

The α-glucosidase inhibitory compound was isolated from *B. martensi Karsch* by the activity-based fractionation using various chromatographies. The isolation procedure was outlined in Fig. 1. The purity of the isolated compound was confirmed by TLC and HPLC. The purified active compound showed a single spot on TLC plate (*vide infra*) and eluted as a single peak (11 min retention time) in HPLC.

Ground bodies of *B. martensi Karsch*
- MeOH extraction
- Concentrated *in vacuo* to dryness

Residues
- Extracted with n- butanol

Butanol extract
- Concentrated
- Silica gel column chromatography
- eluted with butanol:methanol:water(4:1:2)

Active fractions
- Sephadex LH-20 gel filtration
- TLC(n-propanol:1N NH_4OH=7:3)
- HPLC C_{18} reverse phase
- CH_3CN:H_2O (28:72) isocratic system
- UV detection at 206nm

The active compound

Fig. 1. Isolation procedure of the α-glucosidase inhibitory compound from the ground body of *B. martensi Karsch*.

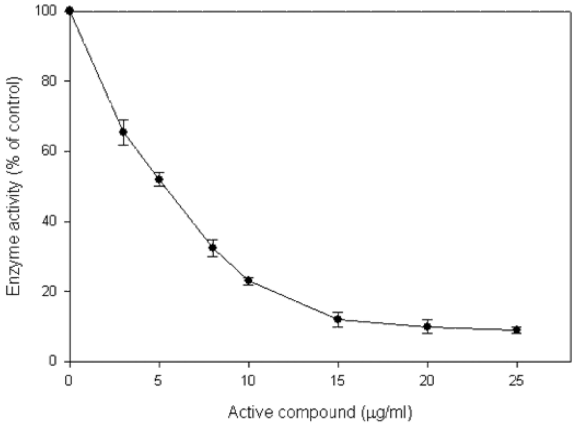

Fig. 2. Effect of the active compound on the activity of α-glucosidase enzyme.
Incubation mixtures were as described in the text.

α-glucosidase inhibitory activity

The effect of the active compound concentration on the inhibition of α-glucosidase was tested and clearly demonstrated that it was a potent inhibitor of α-glucosidase, showing 50% inhibition (IC_{50}) at 5.3 μg/m*l* (Fig. 2). IC_{50} value was obtained from three independent experiments, each performed in duplicate.

Enzyme and the active compound solution were mixed and incubated at 37°C for a 1 h period. During the incubation, aliquots were removed at various time intervals

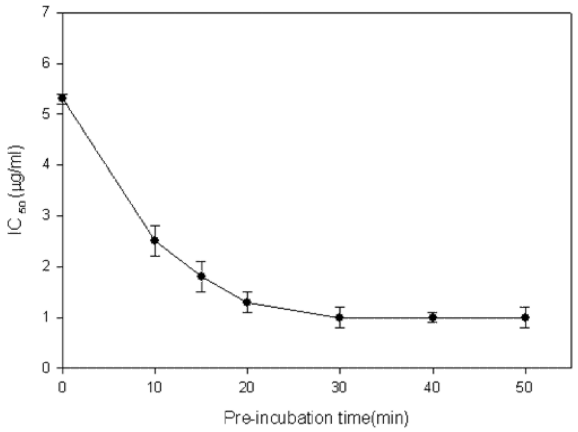

Fig. 3. Effect on α-glucosidase activity of preincubation with the active compound.

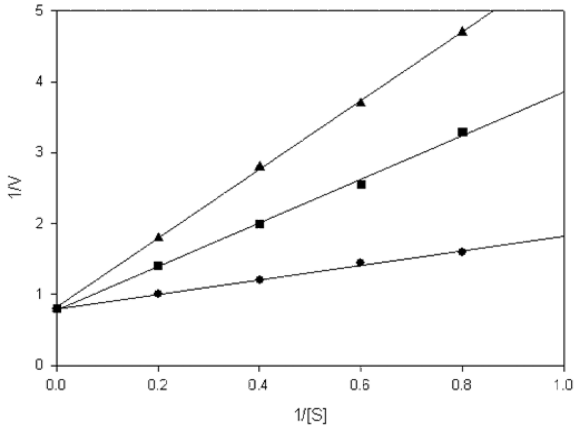

Fig. 4. Effect of substrate concentration on the inhibition of α-glucosidase.
Reaction mixtures were so described in the text but contained various amounts of substrate. Two different concentration of the active compound, 5 μg/m*l* and 10 μg/m*l* were added. The data were plotted by the method of Lineweaver and Burk.
-●- control, -■- 5 μg/m*l*, -▲- 10 μg/m*l*.

and assayed for enzyme activity by mixing with substrate solution. In these cases, controls were prepared without substrate to be certain that no color developed at 405 nm from enzyme and inhibitor alone. Inhibition of a-glucosidase activity by the compound was increased by pre-incubation of the compound with the enzyme, indicating that this compound reacted with enzyme slowly (Fig. 3).

In order to determine whether the compound was acting as a competitive or noncompetitive inhibitor of α-glucosidase, a series of experiments were carried out in which the substrate concentration was varied, and several different concentrations of the compound were used. When the data from these experiments were plotted by the method of Lineweaver and Burk, the intercept of 1/v versus 1/s was the same in the presence or absence of the compound, clearly indicating that the inhibition was of the competitive type with regard to p-nitropheny-α-D-glucopyranoside substrate (Fig. 4).

Table 1. The physico-chemical properties of the active compound from *B. martensi Karsch*

Appearance	white powder
U.V in MeOH	End absorption
Solubility	soluble : MeOH, acetone, EtoAc, $CHCl_3$ slightly soluble : hexane insoluble : H_2O, benzene
Rf value	0.63 silica gel TLC n-propanol: 10% NH_4OH (2 : 1)
Color reaction	positive : antimony chloride orcinol ferric chloride ninhydrin negative : Dragendorff reagent Aniline-diphenylamine Ehrlich reagent

The physico-chemical properties of the active compound

The physico-chemical properties of the compound were summarized in Table 1. The compound was soluble in methanol, acetone, ethylacetate and chloroform, sparingly in hexane, and almost insoluble in water and benzene. In TLC on silica gel 60 F_{254} with n-propanol : 1N NH_4OH (7 : 3) as the solvent, the R_f value was 0.63. Spraying the plates with orcinol ferric chloride and antimony chloride reagent gave violet and yellow spot, respectively, indicating that the compound might be glycoside. The UV spectrum of the compound in methanol exhibited end absorption.

Enzyme inhibitors have potential value in many areas of disease control and treatment. The control of kinetics of carbohydrate digestion and monosaccharide absorption could be of value in the prevention and treatment of diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia (Murai *et al*., 2002). In this respect, inhibitor of α-glucosidase, a typical exo-type amylolytic hydrolase that releases α-glucose from the non-reducing end of polysaccharide and oligosaccharide, are of particular interest. In addition, a wide range of glucosidases are involved in the biosynthesis of the oligosaccharide portions of glycoproteins and glycolipids which play vital roles in cellular structure and function. Many α-glucosidase inhibitors

have been found to possess antiviral activity because of their potential to inhibit the processing of the *N*-linked oligosaccharides on the envelope glycoproteins (Van den Broek *et al.*, 1996). 1-Deoxynojirimycin, castanospermine and their several derivatives were reported to inhibit the HIV replication (Taylor *et al.*, 1991; Papandreou *et al.*, 2002). These principles are the basis for the potential use of the glucosidase inhibitors in the development of new drugs not only for the management of diabetes but also for the viral diseases.

About 50 years have passed since nojirimycin, a glycosidase inhibitor, was discovered from the culture broth of the *Streptomyces* species. Since then, more than 100 glycosidase inhibitors have been isolated from plants and microorganisms. However, this is the first report to find the α-glucosidase inhibitory compound from the Asian scorpion, *B. martensi Kirsch*, which has been regarded as an elixir in oriental medicine. In general, scorpion venoms of *Centruroides noxius*, *Leiurus quinquestriatus*, *Tityus serrulatus* and *Androctonus australis* are very toxic and can be lethal to human (Becerril *et al.*, 1993). However, *B. martensi Karsch*, a widely distributed scorpion species in Asia, is not only less toxic and rarely causes death, but quite contrarily, is used for disease prevention and therapy. Whole scorpions, scorpion tails or their extract have been known to be effective for treating neural diseases, such as incomplete paralysis and mimetic paralysis (Ji *et al.*, 1996). Also, *B. martensi Karsch* has been widely used in the treatment of some immune related disease. Its extract was reported to exert anti-inflammatory effects related to the inhibition of neutrophil functions and of nitric oxide and prostaglandin E2 production (Kim *et al.*, 2005). However, little is still known about the active principles of *B. martensi Karsch* and its pharmacological mechanisms remains to be clarified.

References

Asano, N. (2003) Glycosidase inhibitors: Update and perspectives on practical use. *Glycobiology.* **13**, 93R-104R.

Becerril, B., A. Vazquez, C. Garcia, M. Corona, F. Bolivar and L.D. Possani (1993) Cloning and characterization of cDNAs that code for Na^+-channel blocking toxins of scorpion Centruroides noxius Hoffmann. *Gene* **128**, 165-171.

Chapel, C., C. Garcia, P. Roingeard, N. Zitzmann, J. Dubuisson, R. A. Dwek, C. Tre´ po, F. Zoulim and D. Durante (2006) Antiviral effect of a-glucosidase inhibitors on viral morphogenesis and binding properties of the hepatitis C virus-like particles. *J. Gen. Vir.* **87**, 861-871.

Elbein, A. (1987) Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. *Ann. Rev. Biochem.* **56**, 497-534.

Fischer, P., M. Collin, G. Karlsson, W. James, T. Butters, S. Davis, S. Gordon, R. Dwek and F. Platt (1995) The a-glucosidase inhibitor, N-butyldeoxynojirimycin inhibits human immunodeficiency virus entry at the level of post-CD4 binding. *J. Vir.* **69**, 5791-5797.

Jacob, G. (1995) Glycosylation inhibitors in biology and medicine. *Curr. Opin. Struct. Biol.* **5**, 605-611.

Ji, Y., P. Mansuelle, S. Terakawa, C. Kopeyan, N. Yanaihara, K. Hsu and H. Rochat (1996) Two neurotoxins (BMK I and BMK II) from the venom of the scorpion *Buthus Martensi Karsch:* Purification, amino acid sequences and assessment of specific activity. *Toxicon* **34**, 987-1001.

Kim, K.S., H. S. Cho, S.D. Lee, K. H. Kim, J. Y. Cho, K. H. Chung, Y. C. Lee, S. K. Moon and C. H. Kim (2005) Inhibitory effect of *Buthus martensi Karsch* extracts on interleukin-1b-induced expression of nitric oxide (NO) synthase and production of NO in human chondrocytes and LPS-induced NO and prostaglandin E2 production in mouse peritoneal macrophages. *Toxicology in vitro* **19**, 757-769.

Kornfeld R. and S. Kornfeld (1985) Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54**, 631-664.

Martin, J. L., K. Veluraja, L. N. Ross, G. Johnson, W. J. Fleet, N. G. Ramsden, I. Bruce, M. G. Orchard, N. G. Oikonomakos and A.C. Papageorgiou (1991) Glucose analogue inhibitors of glycogen phosphorylase: design of potential drugs for diabetes. *Biochemistry* **30**, 10101-10116

Mehta, A., N. Zitzmann, P.M. Rudd, T.M. Block and R.A. Dwek (1998) Alpha-glucosidase inhibitors as potential broad based anti-viral agents. *FEBS Letters* **430**, 17-20.

Murai, A., K. Iwamura, M. Takada, K. Ogawa, T. Usui and J. Okumura (2002) Control of postprandial hyperglycaemia by galactosyl maltobionolactone and its novel anti-amylase effect in mice. *Life Sci.* **71**, 1405-1415.

Papandre, M.J., RY. Barbouche, R. E. Guieu, M. Paulekieny and E. Fenouillet (2002) The α-Glucosidase Inhibitor 1-Deoxynojirimycin Blocks Human Immunodeficiency Virus Envelope Glycoprotein-Mediated Membrane Fusion at the CXCR4 Binding Step. *Mol Pharmacol.* **61**,186-193.

Sunkara, P., T. Bowlin, P. Liu and A. Sjoerdsma (1987) Antiretroviral activity of castanospermine and deoxynojirimycin, specific inhibitors of glycoprotein processing. *Biochem. Biophys. Res. Commun.* **148**, 206-210.

Taylor R. and A.B. Johnson (1996) Does suppression of postprandial blood glucose excursions by the alpha-glucosidase inhibitor miglitol improve insulin sensitivity in diet-treated type II diabetic patients? Diabetes Care, **19**, 559-563, 1996.

Tifft, C.J. and R. Proia (2000) Stemming the tide: glycosphingolipid synthesis inhibitors as therapy for storage diseases. *Glycobiology* **10**, 1249-1258.

Van den Broek, L.A., M.W. Kat-Van Den Nieuwenhof, T.D. Butters, C.A. Van Boeckel (1996) Synthesis of alpha-glucosidase I inhibitors showing antiviral (HIV-1) and immunosuppressive activity. *J. Pharm Pharmacol.* **48**, 172-178

Watson, A., G. Fleet, N. Asano, R. Molyneux and R. Nash (2001) Polyhydroxylated alkaloids-natural occurrence and therapeutic applications. *Phytochemistry* **56**, 265-295